Heat shock and thermotolerance of *Escherichia coli* O157:H7 in a model beef gravy system and ground beef

V.K. JUNEJA, P.G. KLEIN AND B.S. MARMER. 1998. Duplicate beef gravy or ground beef samples inoculated with a suspension of a four-strain cocktail of *Escherichia coli* O157:H7 were subjected to sublethal heating at 46 °C for 15–30 min, and then heated to a final internal temperature of 60 °C. Survivor curves were fitted using a linear model that incorporated a lag period (T_L), and D-values and 'time to a 4D inactivation' (T_{4D}) were calculated. Heat-shocking allowed the organism to survive longer than non-heat-shocked cells; the T_{4D} values at 60 °C increased 1·56- and 1·50-fold in beef gravy and ground beef, respectively. In ground beef stored at 4 °C, thermotolerance was lost after storage for 14 h. However, heat-shocked cells appeared to maintain their thermotolerance for at least 24 h in ground beef held at 15 or 28 °C. A 25 min heat shock at 46 °C in beef gravy resulted in an increase in the levels of two proteins with apparent molecular masses of 60 and 69 kDa. These two proteins were shown to be immunologically related to GroEL and DnaK, respectively. Increased heat resistance due to heat shock must be considered while designing thermal processes to assure the microbiological safety of thermally processed foods.

INTRODUCTION

Escherichia coli O157:H7 has become a pathogen of concern to the food industry since documentation of its association with several serious outbreaks of food-borne illness (Mermelstein 1993). The organism is associated with a variety of clinical manifestations in humans, the most common being haemorrhagic colitis. The symptoms of haemorrhagic colitis include severe abdominal pain and bloody diarrhoea. These symptoms may be followed by life threatening complications of haemolytic uremic syndrome and thrombotic thrombocytopenic purpura which contribute to a high mortality rate, particularly in young children and the elderly (Tarr 1994).

While *E. coli* O157:H7 outbreaks have been primarily associated with undercooked ground beef (Pandhye and Doyle 1992), extensive studies by a number of researchers have shown that the organism possesses no unusual heat resistance (Doyle and Schoeni 1984; Line *et al.* 1991; Pandhye and Doyle 1992; Ahmed *et al.* 1995). However, environmental stresses can increase the bacterium's heat resistance (Murano and Pierson 1992; Lou and Yousef 1996). Therefore, the influence of environmental stresses must be considered in the design of thermal processes that provide an adequate degree of protection against *E. coli* O157:H7 or any other food-borne pathogen. The stress associated with a sublethal heat shock, which induces the rapid synthesis of a specific set of proteins known as heat-shock proteins, has recently received considerable attention primarily because of the significance of heat treatment to food safety.

Microbial cells in which heat-shock proteins are synthesized acquire enhanced thermal tolerance to a second heat challenge that would normally be lethal to them. Generally speaking, 'heat-shocked' cells have to be heated twice as long as 'non-heat-shocked cells' to achieve the same extent of lethality (Farber and Brown 1990). In addition to being studied extensively in broth systems, heat-shock response and induced thermal tolerance have been shown to have significance in foods (Mackey and Derrick 1987; Farber and Brown 1990; Shenoy and Murano 1996). These studies on

heat-shocked pathogens in foods have primarily been conducted on *Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica*; there appears to be a lack of published literature on the thermotolerance of *E. coli* O157:H7 in meat following heat shock.

Accordingly, in the work reported here, the objectives were to determine: (a) the effect of heat-shocking *E. coli* O157:H7 inoculated in a model beef gravy system, as well as ground beef, on its thermotolerance and the levels of two heat-shock proteins, GroEL and DnaK, in these cells; and (b) the persistence of the thermotolerance at 4, 15 and 28 °C after heat shock.

MATERIALS AND METHODS

Ground meat

Raw ground beef (93% lean) was obtained from a local retail market and frozen (−18 °C) until use (within approximately 60 days). Prior to inoculation with a four-strain mixture of *Escherichia coli* O157:H7, the meat was thawed at 4 °C over a 24 h period.

Beef gravy formulation

The model beef gravy used in the present study consisted of 1·5% proteose peptone, 5·0% beef extract, 0·5% yeast extract and 1·7% soluble starch. All ingredients were obtained from Difco Laboratories (Detroit, MI, USA). The gravy was sterilized by autoclaving prior to use.

Cultures and preparation of inoculum

The four strains of *E. coli* O157:H7 used throughout this study included 45753–35, 933, A9218-C1 and ent C9490 (Jack-in-the-Box). Strains 45753–35 and 933 are meat and kidney isolates, respectively, and were originally obtained from the Food Safety and Inspection Service, Beltsville, MD. Strains A9218-C1 and ent C9490 are the clinical isolates and were originally obtained from CDC, Atlanta, GA. These strains, maintained as frozen (−70 °C) stocks in brain heart infusion broth (BHI; Difco) supplemented with 10% glycerol, were obtained from our in-house culture collection. During the course of the study, individual stock cultures were maintained on BHI slants at 4 °C with monthly transfers to maintain their viability. To prepare the cell suspensions, a 10 μl loop of stock culture was transferred to 50 ml BHI in 250 ml flasks and incubated at 37 °C for 24 h. After two consecutive transfers using 0·1 ml inocula, final cultures were harvested by centrifugation (5000 *g*, 15 min) at 4 °C and washed twice in 0·1% peptone water (w/v). The cell pellets were resuspended in 10 ml peptone water. The population density in each inoculum suspension was enumerated by spiral plating (Spiral Biotech, Bethesda, MD, USA; Model D) appropriate dilutions (in 0·1% peptone water) in duplicate, on tryptic soy agar (TSA) plates which were then incubated at 37 °C for 24 h. Thereafter, 2 ml cultures of each strain were combined in a sterile test tube, mixed thoroughly, and this cocktail of strains was used for inoculation of meat. Serial dilutions were made in peptone water to obtain the desired cell density before inoculation.

Sample preparation and inoculation

Duplicate 3 g ground beef samples were aseptically weighed into 15 × 22·9 cm sterile whirl-pak sampling bags (Model B736, NASCO, Modesto, CA, USA). The bacterial inoculum (0·1 ml g^{-1}) was added to each bag containing ground beef or to 10 ml beef gravy (0·1 ml inoculum) in sterile test tubes to give a final concentration of approximately 7–8 $\log_{10}$ cfu g^{-1} or ml^{-1}. The test tubes containing beef gravy were vortexed, while the bags containing ground beef were manually mixed to ensure even distribution of the organisms, compressed into a thin layer by pressing against a flat surface, and heat-sealed after excluding as much air as possible.

Heat shock and thermal inactivation

The *E. coli* O157:H7 inoculated in ground beef was heat-shocked by fully submerging each bag in a 46 °C temperature controlled water bath for 15, 25 or 30 min. After heat-shocking, the meat samples were immediately cooled in ice water. Thereafter, the samples were submerged in a 60 °C temperature controlled water bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, NH, USA). The temperature was continuously monitored by two copper-constantan thermocouples inserted at the centre of two uninoculated bags. The thermocouple readings were measured and recorded using a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, MA, USA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Come-up times, which were negligible, were included as part of the total heating time when these were used to calculate the D-values at 60 °C. Two bags for each replicate were removed at designated time intervals. After heating, the bags were plunged into a crushed-ice bath. Negative controls included bags containing uninoculated meat and non-heat-shocked inoculated meat samples. For *E. coli* O157:H7 suspended in beef gravy, the cells were heat-shocked at 46 °C for 15, 25 or 30 min, cooled, and then heated at 60 °C using a submerged coil heating apparatus (Cole and Jones 1990). Samples (0·2–1·0 ml) were removed at pre-determined time intervals and rapidly cooled in melting ice.

In a series of other experiments, ground beef or beef gravy

samples containing *E. coli* O157:H7 were heated at 46 °C for 25 min to heat-shock the organism and subsequently stored at 4, 15 or 28 °C for 6, 8, 14, 24, 27, 30 or 48 h. After each storage period, the samples were heated at 60 °C to determine the duration at different temperatures that induced thermotolerance was maintained.

Enumeration of survivors

To determine the number of surviving cfu g^{-1}, the inoculated beef gravy was serially diluted in 0·1% peptone water (w/v) and surface-plated (appropriate dilutions) onto TSA using a spiral plater. The bags containing the inoculated ground meat samples were opened and sterile 0·1% peptone water (3 ml) was added and the samples stomached for 1 min. The meat slurry was then serially diluted in 0·1% peptone water and surface plated using a spiral plater. When necessary, 0·1 ml of the undiluted suspension was also surface plated. The TSA plates were overlaid with 10 ml of Sorbitol MacConkey agar (pre-tempered to 47 °C; SMA, Oxoid) after 2 h of resuscitation at room temperature to allow recovery of heat-damaged cells (McCleery and Rowe 1995). Plates were incubated at 37 °C for 48 h before counting typical *E. coli* O157:H7 colonies. Isolates from plates were randomly selected and subjected to serological confirmation as *E. coli* O157:H7 serotype (RIM, *E. coli* O157:H7 Latex test; Remel, Lenexa, KS, USA). For each replicate experiment performed in duplicate, an average cfu g^{-1} of four platings at each sampling point was used to determine the D-values.

Survivor curves

Survivor curves were generated by fitting the data to the linear function that allows for the presence of a lag period before initiation of an exponential decline in population density (Buchanan *et al.* 1993; Buchanan *et al.* 1994).

$$Y = Y_0 \quad \text{for} \quad T \leqslant T_L$$

$$Y = Y_0 + m\,(T - T_L) \quad \text{for} \quad T \geqslant T_L$$

where: $Y = \log_{10}$ count of bacteria at time T [$\log_{10}$ (cfu ml^{-1})]; $Y_0 = \log_{10}$ count of bacteria at time $T = 0$ [$\log_{10}$ (cfu ml^{-1})]; m = slope of the survivor curve [$\log_{10}$ (cfu ml^{-1})/min]; T = time (min); and T_L = duration of lag period to initiation of inactivation (min). The curves were fitted using ABACUS, a non-linear curve fitting program that employs a Gauss-Newton iteration procedure. D-values (time to inactivate 90% of the population) were calculated as the negative reciprocal of m, and 'T_{4D}' (time to a 4D inactivation) were calculated using the equation:

$$T_{4D} = T_L + 4 \times D \qquad (1)$$

Statistical analysis

The heat resistance data were analysed by analysis of variance (ANOVA) using SAS (SAS 1989) to determine if there were statistically significant differences among the treatments. The Bonferroni mean separation test was used to determine significant differences ($P < 0{\cdot}05$) among means (Miller 1981).

Analysis of heat-shock proteins

To monitor the increased accumulation of heat-shock proteins in *E. coli* O157:H7 cells following a heat shock, the cultures were centrifuged and washed twice (as above) and the pelleted cells resuspended in 10 ml of pre-warmed (46 °C) beef gravy media and heat-shocked for 15 min at 46 °C. In addition, for one sample, all four strains were mixed together and heat-shocked as a cocktail. To examine the effect of storage following a heat shock on the maintenance of the heat-shock proteins, cells of the four-strain cocktail were heat-shocked for 25 min at 46 °C in beef gravy media. Following the heat shock, the cells were either processed immediately for protein analysis or stored for 48 h at 4, 15 or 28 °C, after which time the cells were harvested. For protein analysis, 1·0 ml aliquots from each sample were collected and centrifuged; 1 ml aliquots from cells that had not been heat-shocked were also harvested to serve as a negative control. The cells were resuspended in 200 μl of 1 × SDS-PAGE sample buffer (Sambrook *et al.* 1989), boiled for 5 min and centrifuged for 2 min in a microcentrifuge. Protein concentration in each sample was determined using the Bicinchoninic Acid protein assay kit according to the manufacturers' directions (Pierce, Rockford, IL, USA). The samples were separated on 10% SDS-polyacrylamide gels on an equal protein basis. Following electrophoresis, the gels were either silver-stained or electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes according to the method of Towbin *et al.* (1979). Non-specific binding sites on the membranes were blocked by incubating in tris-buffered saline (TBS) containing 5% (w/v) non-fat dry milk for 1 h. The membranes were incubated in antibody buffer (TBS-Tween plus 5% non-fat dry milk) containing mouse monoclonal antibodies specific for '*E. coli*' GroEL and DnaK (StressGen Biotechnologies Corp., Victoria, British Columbia, Canada) for 2·5 h. Following washing, the membranes were incubated in antibody buffer containing goat antimouse IgG conjugated with the enzyme alkaline phosphatase for 1 h, washed extensively, and the heat-shock proteins detected colorimetrically using NBT and BCIP according to the manufacturers' instructions (Promega Corp., Madison, WI, USA).

RESULTS

Figure 1 depicts the destruction of *E. coli* O157:H7 in ground meat at 60 °C. The data are expressed as the log of the ratio of count at time t (N) and initial count (N_0), which was calculated by subtracting the log initial count before cooking (log N_0) from the log final count after cooking (log N). The resulting data yielded the log numbers of *E. coli* O157:H7 colonies per gram of beef destroyed by the heat treatment. For non-heat-shocked samples of ground beef heated at 60 °C, the *E. coli* O157:H7 cell population exhibited a linear decline; the count decreased by 2·17 logs (8·55 $\log_{10}$ cfu g^{-1} to 6·38 $\log_{10}$ cfu g^{-1}) within 4 min and by 7·8 logs (< 1 $\log_{10}$ cfu g^{-1}) after 15 min of heating (Fig. 1). In comparison to non-heat-shocked ground beef, exposing ground beef containing *E. coli* O157:H7 to 46 °C for 15 or 30 min prior to heating at 60 °C resulted in deviations in the inactivation kinetics from the log-linear decline in surviving cells with time. After heat-shocking, *E. coli* O157:H7 cells exhibited lag periods or shoulders, where the population remained constant, followed by a linear decline. Also, a subpopulation of cells was observed that declined at a slower rate. Heating heat-shocked *E. coli* O157:H7 cells at 60 °C for 4 min resulted in 0·36 and 0·33 log reductions in *E. coli* O157:H7 colony counts per gram from an initial inoculum of 8·62 and 8·41 $\log_{10}$ cfu g^{-1}, respectively, and after 15 min at 60 °C, the log destruction was 6·62 and 6·17 $\log_{10}$ cfu g^{-1}. Thus, when ground beef containing *E. coli* O157:H7 was heated at 46 °C for 15 or 30 min before exposure to heat at an internal temperature of 60 °C, the heat resistance of the cells was substantially increased and the organism survived longer than non-heat-shocked cells. The T_{4D} values were calculated and compared to combine the effects of T_L and D-values. The T_{4D} values were 1·5-fold higher after heat-shocking (Table 1). Interestingly, the length of the heat shock did not result in a significant difference ($P < 0·05$) in the T_{4D} values.

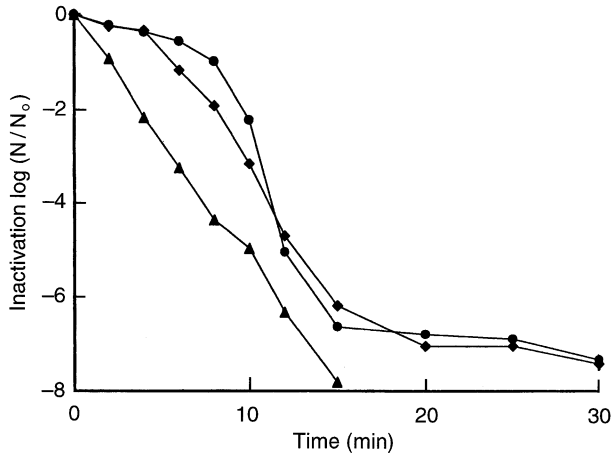

Fig. 1 Destruction of *Escherichia coli* O157:H7 in ground beef at 60 °C. The organism in ground beef was either not heat-shocked or heat-shocked at 46 °C for 15 or 30 min before exposing to 60 °C. The data have been expressed as log of the ratio of count at time t (N) and initial count (N_0). (▲), No heat shock; (●), heat shock, 15 min; (◆), heat shock, 30 min

The mean T_{4D} values at 60 °C for non-heat-shocked and heat-shocked *E. coli* O157:H7 cells suspended in beef gravy were 2·38 min and 3·73 min, respectively (data not shown). Unlike heat-shocked cells in ground beef, the survival curves of heat-shocked cells in beef gravy exhibited a linear decline.

In an additional series of experiments, after heat-shocking (46 °C for 25 min) *E. coli* O157:H7 inoculated in beef gravy or ground beef, the samples were stored for up to 48 h before being heated to a final temperature of 60 °C. The induction of thermotolerance by heat shock was maintained for at least 48 h at 4, 15 or 28 °C in beef gravy (Table 2). In ground beef stored at 4 °C, thermotolerance was lost after storage for 14 h. However, heat-shocked cells appeared to maintain their thermotolerance for at least 24 h in ground beef held at 15 or 28 °C (Table 3).

To determine if the heat-shock treatment used in the present study resulted in an increase in the levels of any of the major heat-shock proteins (Hsp), the protein patterns from control and heat-shocked *E. coli* O157:H7 cells were compared following electrophoresis. Following heat shock of *E. coli* cells in beef gravy for 25 min at 46 °C, the level of one protein with an apparent molecular mass of approximately 60 kDa was significantly increased compared with the level in control cells (Fig. 2A, band marked with asterisk). Upon closer examination of the silver-stained gel, there appeared to be a slight increase in the level of a protein with a molecular mass of approximately 70 kDa in the heat-shocked cells. To determine whether these two proteins detected by SDS-

Table 1 The effect of prior exposure of *Escherichia coli* 0157:H7 in ground beef to 46 °C on the micro-organism's lag period prior to inactivation, D-value and time to a 4D inactivation (T_{4D})[a] at 60 °C

Prior exposure to 46 °C (min)	Lag period (min)	D-value (60 °C) (min)	T_{4D}[b]
Control	0	1·9	7·76[c] ± 0·03
15	6·0	1·8	11·93[d] ± 0·06
25	3·8	1·9	11·55[d] ± 0·01
30	4·6	1·7	11·54[d] ± 0·07

[a]Time to a 4D inactivation.
[b]Mean of two replications, each performed in duplicate and expressed as mean ± standard deviation.
[c,d]Means in the column with different superscript are significantly different ($P < 0·05$).

Table 2 The effect of prior exposure of *Escherichia coli* 0157:H7 in beef gravy to 46 °C followed by storage at 4, 15 or 28 °C for 6 to 48 h on the micro-organism's time to a 4D inactivation $(T_{4D})^{ab}$ at 60 °C

Storage time after heating (h)	T_{4D} (min) at temperatures (°C)[c]		
	4	15	28
6	3·91 ± 0·05	3·44 ± 0·13	3·78 ± 0·18
8	3·76 ± 0·08	3·34 ± 0·00	3·40 ± 0·15
14	3·14 ± 0·03	3·11 ± 0·05	3·39 ± 0·08
24	3·06 ± 0·01	3·17 ± 0·03	4·07 ± 0·03
27	3·31 ± 0·03	3·20 ± 0·08	3·83 ± 0·07
30	3·48 ± 0·03	3·01 ± 0·03	3·67 ± 0·07
48	3·15 ± 0·01	3·29 ± 0·00	3·24 ± 0·06

[a]The T_{4D} of non-heat-shocked cells was 2·38 ± 0·2 and was not significantly altered ($P < 0·05$) after storage at 4, 15 or 28 °C for up to 48 h.
[b]The T_{4D} of cells immediately after heat-shocking was 3·73 ± 0·22 min.
[c]Mean of two replications, each performed in duplicate and expressed as mean ± standard deviation.

Table 3 The effect of prior exposure of *Escherichia coli* 0157:H7 in ground beef to 46 °C followed by storage at 4, 15 or 28 °C for 6 to 48 h on the micro-organism's time to a 4D inactivation $(T_{4D})^{ab}$ at 60 °C

Storage time after heating (h)	T_{4D} (min) at temperatures (°C)[c]		
	4	15	28
6	14·77 ± 0·03	14·15 ± 0·44	19·08 ± 0·19
8	13·57 ± 0·07	15·46 ± 0·02	20·97 ± 0·04
14	13·94 ± 0·09	20·96 ± 0·28	22·32 ± 0·36
24	6·89 ± 0·18	19·49 ± 0·05	17·65 ± 0·10
27	7·78 ± 0·15	7·37 ± 0·18	7·74 ± 0·05
49	7·96 ± 0·19	5·35 ± 0·05	7·62 ± 0·21

[a]The T_{4D} of non-heat-shocked cells was 7·76 ± 0·43 min and was not significantly altered ($P < 0·05$) after storage at 4, 15 or 28 °C for up to 48 h.
[b]The T_{4D} of cells immediately after heat-shocking was 11·55 ± 0·01 min.
[c]Mean of two replications, each performed in duplicate and expressed as mean ± standard deviation.

PAGE analysis corresponded to any of the major *E. coli* Hsps, a Western blot was performed using monoclonal antibodies specific for *E. coli* DnaK (69 kDa) and GroEL (60 kDa). Both of these proteins were detected on the blot in control cells. However, following the heat-shock treatment, the levels of these two proteins increased (Fig. 2B). Densitometric scanning of the blot revealed some strain-to-strain variation in the percentage increase observed in the level of these two proteins. However, on average, the level of GroEL and DnaK increased approximately 30 and 17%, respectively, in the heat-shocked cells. Furthermore, when *E. coli* cells were heat-shocked in beef gravy and then stored for 48 h at 4, 15 or 28 °C, the increase that was observed in the level of GroEL following heat shock was maintained after storage at all three temperatures (Fig. 3), indicating that the induction and maintenance of thermotolerance in these samples correlated with an increase in heat-shock protein level.

DISCUSSION

This study examined the effects of heat-shock and incubation temperature following heat shock on the heat shock-induced thermotolerance of *E. coli* O157:H7 in a model beef gravy system as well as in ground beef. Previous workers have demonstrated the significance of the heat-shock response and induced thermotolerance of *Yersinia enterocolitica* in ground pork (Shenoy and Murano 1996), *Listeria monocytogenes* in a fermented sausage (beef and pork) mix (Farber and Brown 1990) and *Salmonella thompson* in liquid whole egg, reconstituted dried milk or minced beef (Mackey and Derrick 1987). Our findings illustrate the occurrence of the heat-shock phenomenon with *E. coli* O157:H7 in beef gravy and ground beef where T_{4D} values at 60 °C were increased 1·56- and 1·50-fold, respectively, over non-heat-shocked cells. Murano and Pierson (1992) obtained similar results when they used trypticase soy broth as the heating menstruum and reported that the D-values at 55 °C of *E. coli* O157:H7 heat-shocked at 42 °C for 5 min increased by more than twofold in the case of aerobically grown cells, and 1·5-fold when the organism was grown anaerobically, as compared with cells not exposed to a heat shock. In the present study, non-linear inactivation kinetics were observed in beef and not in beef gravy. This may be attributed to differences in composition (more solids in beef) between the two substrates. Farber and Brown (1990) reported no significant increase in thermotolerance of *L. monocytogenes* after a heat shock at 48 °C for 30 or 60 min in a pork/beef mixture whereas a 2·3-fold increase was noted by Linton *et al.* (1990) after a 10 min heat shock at 48 °C in trypticase soy + 0·6% yeast extract broth. These studies, as well as the present study, clearly indicate that the thermotolerance response of bacteria depends upon the heating menstruum during the heat shock. Heat shock, in conjunction with the composition of the heating medium, conferred increased heat resistance on *S. thompson* (Mackey and Derrick 1987). Certainly, it would be difficult to predict the extent of induced heat tolerance in foods based on data obtained in media.

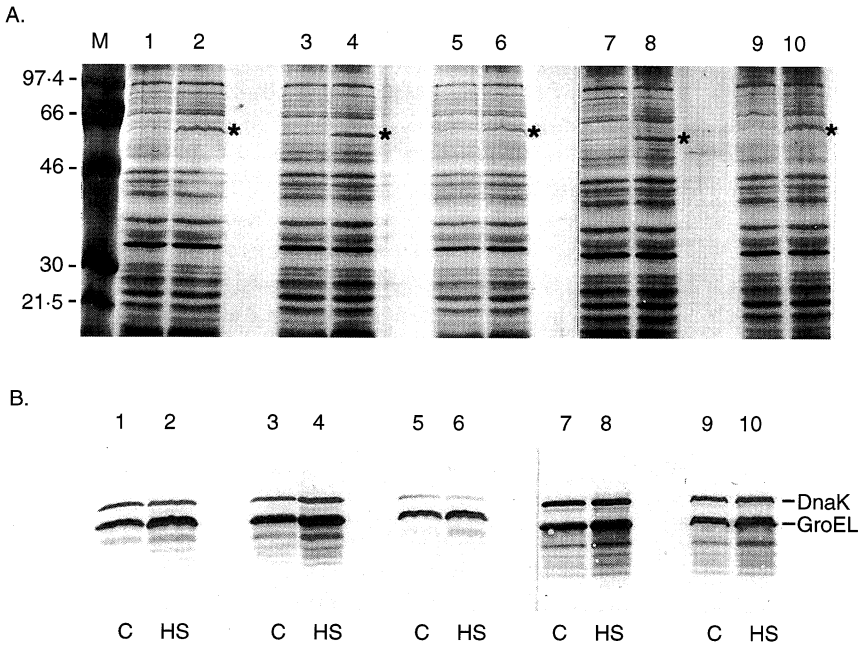

Fig. 2 Accumulation of heat-shock proteins in *Escherichia coli* O157:H7 strains following a 15 min heat treatment. Following electrophoresis, the gel was either silver-stained (A) or electrophoretically transferred to a PVDF membrane for immunoblot analysis (B). Lanes 1 and 2: *E. coli* four-strain cocktail; lanes 3 and 4: strain 45753–35; lanes 5 and 6: strain 933; lanes 7 and 8: strain A9218-C1 and lanes 9 and 10: strain Ent C9490. Molecular weight markers were run in the lane marked M in A. Lanes marked with C represent protein samples from control, non-heat-shocked cells; lanes marked with HS represent protein samples from cells heat-shocked at 46 °C. The asterisks in A mark the position of the 60 kDa GroEL protein

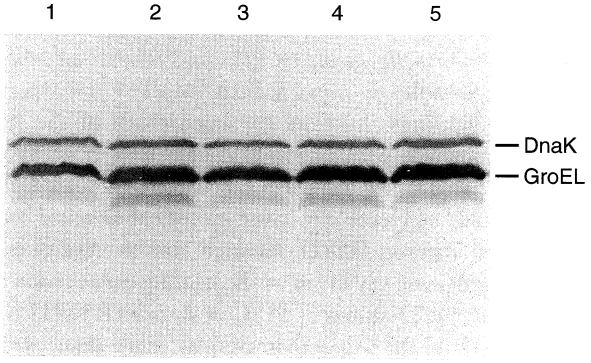

Fig. 3 Maintenance of heat-shock proteins in an *Escherichia coli* O157:H7 four-strain cocktail following a heat shock at 46 °C for 25 min and storage of the cells for 48 h at 4, 15 or 28 °C. Following electrophoresis, the proteins were transferred electrophoretically to a PVDF membrane for immunoblot analysis. Lane 1: control (non-heat-shocked) cells; lane 2: cells heat-shocked for 25 min at 46 °C; lane 3: heat-shocked cells stored at 4 °C; lane 4: heat-shocked cells stored at 15 °C; lane 5: heat-shocked cells stored at 28 °C

As temperature fluctuations are a common occurrence in a food processing environment as well as during transportation, distribution, storage or handling in supermarkets or by consumers, bacterial cells in meat are likely to encounter temperature shifts. Therefore, temperature plays a significant role and must be considered when determining the persistence of heat-shock-induced thermotolerance. In the present study, we performed investigations on post-heat-shocking storage temperatures (4, 15 and 28 °C) and storage times ranging from 6 to 48 h to determine the time and temperature when a decay in thermotolerance occurs in *E. coli* O157:H7 inoculated in beef gravy or ground beef. While heat-shocked *E. coli* O157:H7 cells held at 4, 15 or 28 °C maintained induced thermotolerance for up to 48 h in beef gravy, it was interesting to note that *E. coli* O157:H7 cells in beef lost their thermotolerance after 14 h at 4 °C and after 24 h in beef held at 15 or 28 °C. These findings regarding a longer duration of induced thermotolerance with storage at higher temperatures are not in agreement with other researchers (Jorgensen *et al.* 1996). These researchers, working with *L. monocytogenes* suspended in tryptic phosphate broth, heat-shocked cells at 46 °C for 30 min and held the samples at 4, 10 or 30 °C before heating at 58 °C. Their results indicated that heat-shock-induced thermotolerance was maintained for a longer time at 4 and 10 °C compared with cells stored at 30 °C. According to Lindquist (1986), the persistence of heat-shock-induced thermotolerance appears to be a function of many factors including the temperature at which heat-shocking is done, the previous incubation temperature of the cell, and the metabolic state of the cell. Mackey and Derrick (1986) working with *Salmonella typhimurium* in tryptone soy broth examined the induction of heat resistance in cells after shifting from 37 °C to 42, 45 or 48 °C and their survival after heating at 55 °C for 25 min. These researchers found that the heat resistance of the cells increased rapidly following the temperature shift and persisted at high levels for 10 h when the temperature was maintained and declined thereafter. In a study by Farber and Brown (1990), when a

sausage mix inoculated with 7 $\log_{10}$ cfu g^{-1} of *L. monocytogenes* was initially subjected to a heat-shock temperature of 48 °C for 1 h and then held at 4 °C for 24 h before being heated at a final temperature of 64 °C, a loss of thermotolerance was observed compared with cells which were heat-challenged immediately after heat-shocking. Another study (Katsui *et al.* 1982) showed that the exposure of non-heat-shocked *E. coli* to 0 °C before heating significantly increased the heat sensitivity of the exposed cells. In our previous study, the heat resistance of non-heat-shocked cells of *E. coli* O157:H7 inoculated in ground beef was not altered after storage at 4 °C for 48 h (Juneja *et al.* 1997).

In bacteria, the loss of normal cellular functions that results due to environmental stresses, including a sudden increase in temperature, is recovered by the synthesis of stress proteins such as Hsps. These Hsps are highly conserved among prokaryotic and eukaryotic organisms (Lindquist 1986) and increase the potential of bacteria to withstand severe subsequent stresses. In the present study, it is not surprising to find Hsps in non-heat-shocked cells. In *E. coli*, about 17 heat-shock proteins are found which are diverse with respect to size, net charge, and levels or extent to which they are induced in response to heat shock (Neidhardt *et al.* 1984). Ten of these Hsps are the products of known genes and have been characterized (Neidhardt and VanBogelen 1987). It has been reported that two stress proteins of 60 and 69 kDa correspond to the GroEL and DnaK proteins of *E. coli* (Georgopoulos *et al.* 1990). The 60 kDa (GroEL) protein is involved in the morphogenesis of coliphage and is also essential for *E. coli* growth (Friedman *et al.* 1984; Fayet *et al.* 1989). GroEL has been reported to protect RNA polymerase (RNAP) from heat inactivation and 'resurrects' heat inactivated, aggregated RNAP (Georgopoulos *et al.* 1994). In the present study, the level of the 60 kDa GroEL protein increased significantly following heat shock whereas the increase in the level of the 69 kDa DnaK protein was not as high. Murano and Pierson (1992) reported stress proteins with molecular masses of 71 and 84 kDa in *E. coli* O157:H7 and found that the concentration of these proteins in heat-shocked cells depended upon the growth atmosphere prior to heat shock.

The microbial safety of thermally processed foods depends on ensuring that potential food-borne pathogens likely to be present in foods are killed during heating. Thus, the phenomenon of the heat-shock response and induced thermotolerance is of substantial practical importance to food processors in products that are heated to lower rather than higher temperatures (> 65 °C). Heat-shocking conditions may be encountered in minimally processed, refrigerated foods of extended durability such as *sous-vide* foods, in which there is an increased interest at the present time. Slow heating rate/long come-up times and low heating temperatures employed in the production of *sous-vide* cooked foods expose the microbial cells to conditions similar to heat shock, thereby rendering these cells more thermotolerant. Additionally, thermotolerance may be a concern in meat products kept on warming trays before final heating or reheating or when there is an interrupted cooking cycle during processing due to equipment failure. Thus, increased heat resistance due to heat shock must be considered while designing thermal processes to assure the microbiological safety of thermally processed foods; the heat treatment should be sufficient to inactivate the thermotolerant food-borne pathogens. Refrigeration and rapid heating of the products will limit the development of thermotolerance during pasteurization. The present study has shown that heat-shocking *E. coli* O157:H7 in ground beef can increase the heat resistance of the organism more than 1·5-fold. It is therefore recommended that guidelines be established so that prophylactic measures are adopted and environmental stresses (such as heat) do not render bacteria better able to survive thermal processing procedures that would normally be considered adequate.

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